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In vitro preactivated human T cells engraft in SCID mice and migrate to murine lymphoid tissues

N. ARMSTRONG, F. CIGEL, W. BORCHERDING*, R. HONG* & V. MALKOVSKA *Departments of Medicine and *Immunology, University of Wisconsin, Madison, WI, USA*

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SUMMARY

Mice with severe combined immunodeficiency (SCID) accept grafts of human T and B lymphocytes derived from resting peripheral blood mononuclear cells (PBMC). We wished to determine whether activated human T cells engraft and migrate into lymphoid tissues in SCID mice. PBMC (50×10^6) activated *in vitro* in a 4-day mixed lymphocyte culture (MLC) were injected into the peritoneum of 12 SCID mice. In 11 of 12 animals killed at 3 or 4 weeks after injection, human cells were detected in cells pooled from lymphoid organs by flow cytometry and by immunohistochemical staining of frozen tissue sections. The percentage of $CD45^+$ cells in the 11 mice ranged from 2% to 45% and the absolute numbers of $CD45^+$ cells recovered from lymphoid organs ranged from 4×10^6 to 90×10^6. Up to 93% of the human cells expressed the CD3 antigen together with either CD4 or CD8. Human T cells were localized in periarteriolar areas in murine spleens, whereas in the lymph nodes and gut mucosa, the T cells did not show the pattern for T-dependent areas found in human lymphoid tissue. Numerous human plasma cells were detected in the spleen and gut mucosal crypts of engrafted SCID mice. Human IgG was detected in the serum of all 11 engrafted SCID mice. The functional activity of human T cells recovered from murine splenic tissue was very low 3–4 weeks after engraftment.

Keywords SCID mouse activated human T cells xenotransplantation

INTRODUCTION

The CB.17 scid/scid mice have a severe combined immunodeficiency (SCID) as a result of their inability to correctly rearrange the immunoglobulin and T cell receptor genes [1–3]. Most SCID mice have no detectable B and T lymphocytes because these cell lineages are arrested at an early stage of their development. It has been shown that SCID mice can accept human haematopoietic and lymphoid cell xenografts [4–6]. This prompted the idea that the resulting mouse chimaeras carrying human immune systems could be used as models for investigating the *in vivo* functions of human lymphocytes. Recent applications of the SCID mouse have included the study of certain aspects of autoimmunity [7], HIV infection [8,9], transplantation immunity [10,11], and anti-tumour immune response [12,13].

Two differing approaches to this xenotransplantation of human immunocompetent cells into SCID mice have been successful. McCune *et al.* implanted a source of human haematopoietic stem cells (fetal liver) as well as human fetal thymus and lymph node to provide an environment for T cell homing and differentiation [4]. Several weeks later, mature human T cells of either CD4 or CD8 phenotype were detected in the peripheral circulation [4]. These human T cells responded to stimulation by mitogens and allogenic human cells [14]. Human macrophages were not detected in the chimaeras [14].

A second model, developed by Mosier *et al.* utilized normal human peripheral blood mononuclear cells (PBMC) injected intraperitoneally into SCID mice [5]. The human cells survived in the mice for several months and even increased in numbers [5,15]. Reports vary as to the relative proportions of human T cell subsets, B cells, and monocytes in long-term SCID mouse recipients [5,7,15]. Mosier *et al.* recovered all of the major cell populations present in human PBMC from cell pools obtained from lymphoid tissues, blood, and peritoneal cavities of SCID recipients. Over time, the number of T cells declined while the B cell population increased [5]. In contrast, Tary-Lehman & Saxon detected mostly human T cells, low numbers of B cells, and no monocytes in lymphoid organs of SCID mice inoculated with human PBMC [15]. All investigators using this approach observed a rising titre of human immunoglobulins in SCID mouse serum, indicating successful engraftment and functional activity of human cells [5,7,15]. A specific secondary antibody response could be induced to tetanus toxoid [5].

Although the functional potential of human lymphocytes in SCID mice still needs to be clarified, most studies suggest that the induction of primary T cell responses to antigens in SCID chimaeras will be difficult to achieve [15,16]. It may, however, be feasible to use this model to study *in vivo* functions of human T

Correspondence: V. Malkovska, Department of Medicine, Clinical Sciences Centre, University of Wisconsin-Madison, WI 53792, USA.

cells that have been primed and cultured *in vitro*. Such models could clarify certain aspects of human immune responses against infections, allografts, or cancer [17]. We studied the engraftment and homing of human PBMC activated *in vitro* in a one-way mixed lymphocyte culture (MLC). We show that all mature cell lineages required to constitute a functional immune system in these cultures engrafted in SCID mice and were detected in murine peripheral lymphoid organs using both immunohistochemical methods and flow cytometry. However, the human cells recovered from murine spleen after long-term engraftment showed diminished functional activity.

MATERIALS AND METHODS

Mice

Mutant CB.17 scid/scid (SCID) mice, 6–8 weeks old, were obtained from the Jackson Laboratory. All animals used had total immunoglobulin levels below 10 μg/ml ('non-leaky' SCID mice), as measured by ELISA (see below).

Human cell activation and injection

Human PBMC were obtained by separation of heparinized blood from healthy donors on a Ficoll–Histopaque gradient. The PBMC were washed twice and resuspended in RPMI 1640 supplemented with 25 mM HEPES buffer, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated FCS (this media is designated FCS-RPMI).

The PBMC were stimulated *in vitro* in a one-way MLC as described [4]. Responder PBMC (at a final concentration of 10^6/ml) were cultured with 10^6 irradiated PBMC (3000 cGy) in flat-bottomed microwell plates for 4 days at 37°C (5% CO_2, humid chamber). The cells were then phenotyped using MoAb and flow cytometry (see below). Cells (50×10^6) were injected intraperitoneally into each mouse.

Determination of serum levels of human and mouse immunoglobulin by ELISA

Blood was obtained weekly from SCID mice by bleeding the tail vein. Mouse immunoglobulin was quantified in the serum by a 'sandwich' ELISA [18] using goat anti-mouse immunoglobulin (Sigma) as the capture antibody, pooled mouse serum of known immunoglobulin concentration as the standard, and goat anti-mouse alkaline-phosphatase conjugated antibody (Sigma) as the detection reagent. Carnation Dry Milk (30 mg/ml) was used as the blocking buffer. Microplates (Corning) precoated with the capture reagent were incubated for 3 h at room temperature with dilutions of serum or the standard, washed, and the detection reagent added. Then *p*-nitrophenyl-phosphate (PNP) (Sigma) was added for colour development. The absorbance at 405 nm was quantified on a Dynatech Micro-ELISA microplate reader. Serum immunoglobulin levels were calculated from the standard curve.

For measurement of human immunoglobulins, goat anti-human immunoglobulin (Sigma) was used as the capture reagent, pooled human serum of known immunoglobulin concentration as the standard, and peroxidase-conjugated goat anti-human immunoglobulin (Sigma) as the detection reagent. After peroxidase-catalysed colour development, the absorbance at 490 nm was quantified on a Dynatech Micro-ELISA microplate reader.

Immunofluorescence staining and flow cytometry

Cells were suspended in PBS/BSA (0·2% w/v) and then incubated with the appropriate FITC-conjugated MoAb against human or murine lymphocyte antigens. Anti-human MoAbs were: CD45(HLe1)-FITC, CD2(Leu-5b)-FITC, CD3(Leu-4)-FITC, CD4(Leu-3a+3b)-FITC, CD8(Leu-2a)-FITC, CD16(Leu-11a)-FITC, CD19(Leu-12)-FITC, and CD20(Leu-16)-FITC (all from Becton Dickinson). Goat anti-human IgG1 was used as the isotype control. Anti-mouse MoAbs used were H2K-(d-haplotype)-FITC, Ly5(B220)-FITC, and Thy1.2-FITC (all from PharMingen). Rat anti-mouse IgG1 (Becton Dickinson) was used as the isotype control. MoAb (1 μg/10^6 cells) was incubated for 30 min at 4°C, and washed twice with PBS/BSA solution. Fluorescence intensity was measured using the FACScan flow cytometer. Scatter gates were set on lymphocyte and monocyte cell populations.

Immunohistological staining with biotinylated MoAbs

Tissue samples from SCID mice were frozen at −70°C in embedding medium immediately after collection. Tissue sections were cut at −12°C in a cryostat, air-dried overnight and fixed in 100% acetone for 10 min at room temperature. After rehydration (6 min in PBS), the appropriate biotinylated MoAb (anti-human CD2) (AMAC, Inc.), rat anti-mouse Ly5(B220) (PharMingen), and rat anti-mouse Thy1.2 (PharMingen) was added at 2 μg MoAb/100 μl PBS. The slides were incubated for 1–2 h (25°C, humid chamber). The slides were then washed for 6 min in PBS. Streptavidin-peroxidase (Kirkegaard-Perry Laboratories, Inc.) at 1 μg MoAb/100 μl PBS was added and the slides were incubated for 10 min (25°C, humid chamber) and washed for 10 min in PBS. The Zymed Histostain-SP Basic Kit reagents were used to develop colour and for mounting the sections.

Staining for intracytoplasmic immunoglobulin

Slides were prepared, stored, and fixed as detailed above. Tissue sections were covered with 15 μl of concentrated, FITC-conjugated mouse anti-human MoAbs (anti-human immunoglobulin heavy chain: anti-μ and anti-γ; both courtesy of Dr P. Sondel, Madison, WI), incubated for 30 min (25°C, humid chamber) and washed for 10 min in PBS. Slides were mounted using Quantafluor medium and viewed with a fluorescence microscope.

Histology of SCID mouse tissues

Tissue samples were fixed in 10% formalin solution. The material was then embedded in paraffin, cut serially, and stained with haematoxylin/eosin.

Enrichment for human cells from murine spleens using complement and antibody

Murine spleens were removed under sterile conditions and teased into single-cell suspensions in FCS-RPMI. The murine erythrocytes were lysed with lysing buffer (0·3 M NH_4Cl, 0·02 M $KHCO_3$, 0·8 mM Na_2EDTA in distilled H_2O, sterilized by filtration) for 10 min and then washed twice in FCS-RPMI. Next, the cells were resuspended to 10^6/ml and 50 μl of rat anti-mouse MoAb (M1/42.3.9.8.HLK-antiH2-haplotype, courtesy of Dr D. Paulnock, Madison, WI) per 10^6 cells were added. The cells were then incubated for 1 h at 4°C, washed in FCS-RPMI, and resuspended to 10^6/ml. A second MoAb, goat anti-rat-FITC (Tago, Inc.) was added (1:50, incubate 30 min at 4°C).

Rabbit complement-MA (Cedarlane Laboratories) was then added (final concentration of 1:3–1:5) and the cells were incubated for 1 h at 37°C in a humid chamber. The suspension was washed, layered over FCS, and centrifuged at 300 *g* for 5 min. Then the cells were resuspended in FCS-RPMI and used in the mitogen/antigen DNA synthesis response assays (see below).

Proliferative responses to mitogens and antigens

DNA synthesis of fresh human PBMC or enriched human cells obtained from murine spleens was measured after stimulation with allogenic PBMC or soluble antigens. The cells were resuspended at 2×10^6/ml in FCS-RPMI and then incubated with phytohaemagglutinin (PHA) (Wellcome Diagnostics) at 2 μg/ml, concanavalin-A (Con A) (Calbiochem Corp.) at 20 μg/ml, or bacto-lipopolysaccharide W (LPS) (Difco Laboratories Inc.) at 20 μg/ml. Antigens were candida (Hollister-Stier Laboratories) at 10 μg/ml, tetanus toxoid (Wyeth, Inc.) at 10 μg/ml, streptokinase (KabiVitrum AB) at 25 μg/ml in 96-well round-bottomed microwell plates for 3 days (37°C, 5% CO_2, humid chamber). DNA synthesis was measured by adding 1 μCi of ^{3}H-thymidine and then incubating for 18 h (37°C, 5% CO_2, humid chamber). Filter discs were counted using a beta counter.

RESULTS

Engraftment of activated human cells into SCID mice

Twelve 6–8 weeks old 'non-leaky' SCID mice (serum immunoglobulin levels < 10 μg/ml) were inoculated intraperitoneally with $50–70 \times 10^6$ human PBMC that had been activated *in vitro* in a one-way MLC for 4 days. Control mice were given PBS. The mice were killed and analysed after 3–4 weeks. No macroscopic signs of graft-*versus*-host disease (GVHD) such as hunched back, ruffled fur, emaciation, or diarrhoea were present. On *post-mortem* examination, the xenografted SCID mice had splenomegaly. Significantly higher numbers of cells were recovered from their spleens compared with controls. The size of lymph nodes and thymuses was not significantly different in the two groups. Other organs in the xenografted SCID mice were macroscopically normal.

Sections of liver, skin, and gut mucosa were histologically examined for signs of GVHD. Although low numbers of scattered plasma cells were seen in the spleen, lymph nodes, and gut mucosa, no histological signs of GVHD such as lymphocytic infiltration of the liver, lymphocytic infiltration of the dermis of the skin, or significant lymphocytic infiltration of the intestinal crypts were detected in any of the xenografted SCID mice.

Detection of murine and human immunoglobulin in murine serum

The total murine immunoglobulin levels in the 12 SCID mice used in this experiment ranged from 0 to 6·1 μg/ml before the injection of activated human cells, and did not increase significantly 3–4 weeks later.

Before the injection of human cells, all murine sera tested negative for human immunoglobulins (IgG and IgM) in our ELISA. Three to four weeks after the injection of activated human PBMC, all 12 of the mice studied had measurable human IgG and IgM with or without IgA in their serum (Table 1).

Table 1. Levels of human immunoglobulins in murine serum

SCID mouse*	Serum immunoglobulin† (μg/ml)		
	IgG	IgM	IgA
1	2082	ND	ND
2	276	ND	ND
3	0	0	0
4	1	ND	ND
5	3776	ND	ND
6	1004	621	7
7	879	696	6
8	233	59	1
9	635	146	ND
10	226	42	2
11	55	ND	ND
12	98	ND	5

* Twelve SCID mice were injected intraperitoneally with 50×10^6 *in vitro* mixed lymphocyte culture (MLC)-activated peripheral blood mononuclear cells (PBC) and killed 3–4 weeks after cell transfer.

† Murine serum levels of human immunoglobulins as measured by an ELISA.

ND, Not done.

Analysis of murine lymphoid tissues by flow cytometry

Flow cytometric analysis of MLC-activated human PBMC (before injection into SCID mice) is shown in Table 2. After sacrifice, flow cytometric analysis of lymphoid organs of the xenografted SCID mice was performed using a panel of FITC-conjugated antibodies against human and murine leucocyte antigens (Table 2). Pooled lymphocytes from spleens and lymph nodes of control SCID mice were negative for CD45 or other human antigens (data not shown). In contrast, 10/12 SCID mice receiving MLC-activated human PBMC contained $CD45^+$ cells in their pooled lymphoid tissues at 3 or 4 weeks. The numbers of human cells, expressed as the percentage of $CD45^+$ cells or as the absolute number of live $CD45^+$ cells in pooled murine lymphoid tissues, ranged from 2% to 45% and from 4×10^6 to 90×10^6 cells, respectively. Analysis of human lymphocyte subsets was performed in nine of the 10 mice that had detectable $CD45^+$ cells. As shown in Table 2, most of the engrafted human cells were T lymphocytes ($CD3^+$). In all but one animal, the rest of the $CD45^+$ cells were monocytes, as suggested by their light scatter characteristics and expression of the CD14 antigen. Lymphoid cells from one engrafted SCID mouse contained significant numbers of human B lymphocytes (5% of CD19 and 3% of $CD20^+$ cells). Human lymphocytes expressing the NK marker CD16 were detected at low levels (1%) in one mouse only.

Analysis of murine lymphocyte antigens in the xenotransplanted SCID mice showed a small percentage of $Thy1.2^+$ and $B220^+$ cells (Table 2) that was not significantly different from controls (data not shown).

Table 2. Flow cytometric analysis of human and murine lymphocyte antigens on *in vitro* mixed lymphocyte culture (MLC)-activated human peripheral blood lymphocytes before and after engraftment into SCID mice

Human markers*	MLC-activated human PBMC† (%)	Cells from pooled murine lymphoid organs‡ (%)
CD45	98	2–45
CD3	67	2–42
CD4	43	2–38
CD8	19	1–16
CD14	13	1–12
CD16	16	0–1
CD19	13	0–5
CD20	11	0–4
CD25	ND	<1
Murine markers§		
H2	0	58–98
B220	0	1–3
Thy1.2	0	1–8

* Monoclonal antibodies specific for human cell surface markers.

† See Materials and Methods for technique.

‡ Cells obtained from xenografted SCID mouse spleen, blood, and lymph nodes were pooled and stained with FITC-labelled MoAbs and analysed by flow cytometry. The range of values for the 11 mice which tested positive for human antigens is shown.

§ Monoclonal antibodies against murine lymphocyte antigens.

Immunohistochemical analysis of SCID mouse organs using biotinylated MoAbs

Immunohistochemical staining with biotinylated MoAb against human CD2 was used to demonstrate the histological localization of human T lymphocytes in lymphoid tissues of the xenografted SCID mice. $CD2^+$ tissue staining was found in all animals in which the presence of human T cells had been previously demonstrated by flow cytometry. In the splenic tissue, most human T cells were localized in continuous areas surrounding the blood vessels (Fig. 1a). Only small numbers of isolated $CD2^+$ cells were found scattered elsewhere in the splenic red pulp (Fig. 1a). Staining with biotinylated B220 and Thy1.2 MoAb revealed only few positive cells which was in agreement with the flow cytometric analysis.

In the lymph nodes of xenografted SCID mice, varying numbers of $CD2^+$ cells were scattered throughout the nodes. These cells did not appear to accumulate in recognizable 'T-dependent' areas (Fig. 2a). As in splenic tissues, the staining with biotinylated B220 MoAb (Fig. 2b) and Thy1.2 MoAb (data not shown) demonstrated only small numbers of positive cells. The staining of gut tissue from xenografted SCID mice with biotinylated CD2 MoAb revealed occasional positive cells, either single or in small clusters, in the subepithelial layer of the intestinal crypts (data not shown). All tissue sections from control SCID mice stained negative with biotinylated antibodies against human CD2 (data not shown).

Demonstration of human immunoglobulin-secreting cells in SCID mouse organs

Histological examination of the spleens from the mice that received human PBMC showed variable numbers of lymphoid cells with prominent nucleoli organized in perivascular areas. Plasma cells were seen in the same areas (Fig. 3). The spleens of control mice contained only haematopoietic progenitors without lymphocytes or plasma cells. As mentioned above, all 12 SCID mice had detectable human IgG, IgM and in some cases, IgA in their serum by 3 weeks after the injection of activated human PBMC. Histological staining of their lymphoid organs demonstrated the presence of plasma cells which were absent in control animals. To test whether these plasma cells were of human origin, selected samples of spleen, lymph node, thymus and gut mucosa were stained with FITC-conjugated anti-human immunoglobulin heavy-chain anti-μ and anti-γ MoAb. Spleen, lymph nodes, and gut from control SCID mice were used as negative controls. As shown in Fig. 3a, b, cells with strong cytoplasmic staining for human IgG or IgM were seen either singly or in clusters in murine lymph nodes and spleens and the same organs also contained morphologically identifiable plasma cells. In spleen samples, the human IgG^+ or IgM^+ cells were localized mainly in the perivascular lymphoid areas where the $CD2^+$ cells had been visualized. In the samples of gut mucosa from three engrafted SCID mice, only staining for intracytoplasmic human IgG was positive. The human plasma cells were localized in the lamina propria of the gut epithelium.

In vitro *proliferative response of human PBMC derived from SCID mouse spleen*

Splenic lymphocytes from two SCID mice showing high numbers of engrafted human cells were depleted of murine cells as detailed above. The lymphocytes (containing >95% $CD45^+$ cells) were then tested for proliferative responses against tetanus toxoid, candida antigen, streptococcal antigen, and allogenic lymphoid cells. There was no significant increase in DNA synthesis (as measured by incorporation of ^{3}H-thymidine) in response to any of these antigens. Human cells obtained from one mouse showed a small increase in ^{3}H-thymidine uptake in response to the original MLC stimulator cells (3923 $\pm$ 999 ct/min) as compared with autologous human cell control (1287 $\pm$ 243 ct/min).

DISCUSSION

The variation encountered in numbers of $CD45^+$ cells recovered from transplanted SCID mice concords with studies using non-activated human PBMC for xenotransplantation. Krams *et al.* found that 0–56% of SCID splenic lymphocytes were $CD3^+$ [7]. Tary-Lehman & Saxon found that 0–57% of SCID splenic lymphocytes were $CD45^+$ [15]. Mosier *et al.* found less variation, with human cells accounting for between 80% and 92% of SCID splenic lymphocytes [5]. The variability of the engraftment might be explained by the presence of murine T and B cells ('leakiness') in some of the animals. Although we selected 'non-leaky' SCID mice for the experiment, it could be that activated human PBMC and/or their products could induce differentiation of murine lymphocytes. However, we found no evidence for 'leakiness' at the time the mice were killed. Only very low numbers of $Thy1.2^+$ or $B220^+$ cells were detected in murine

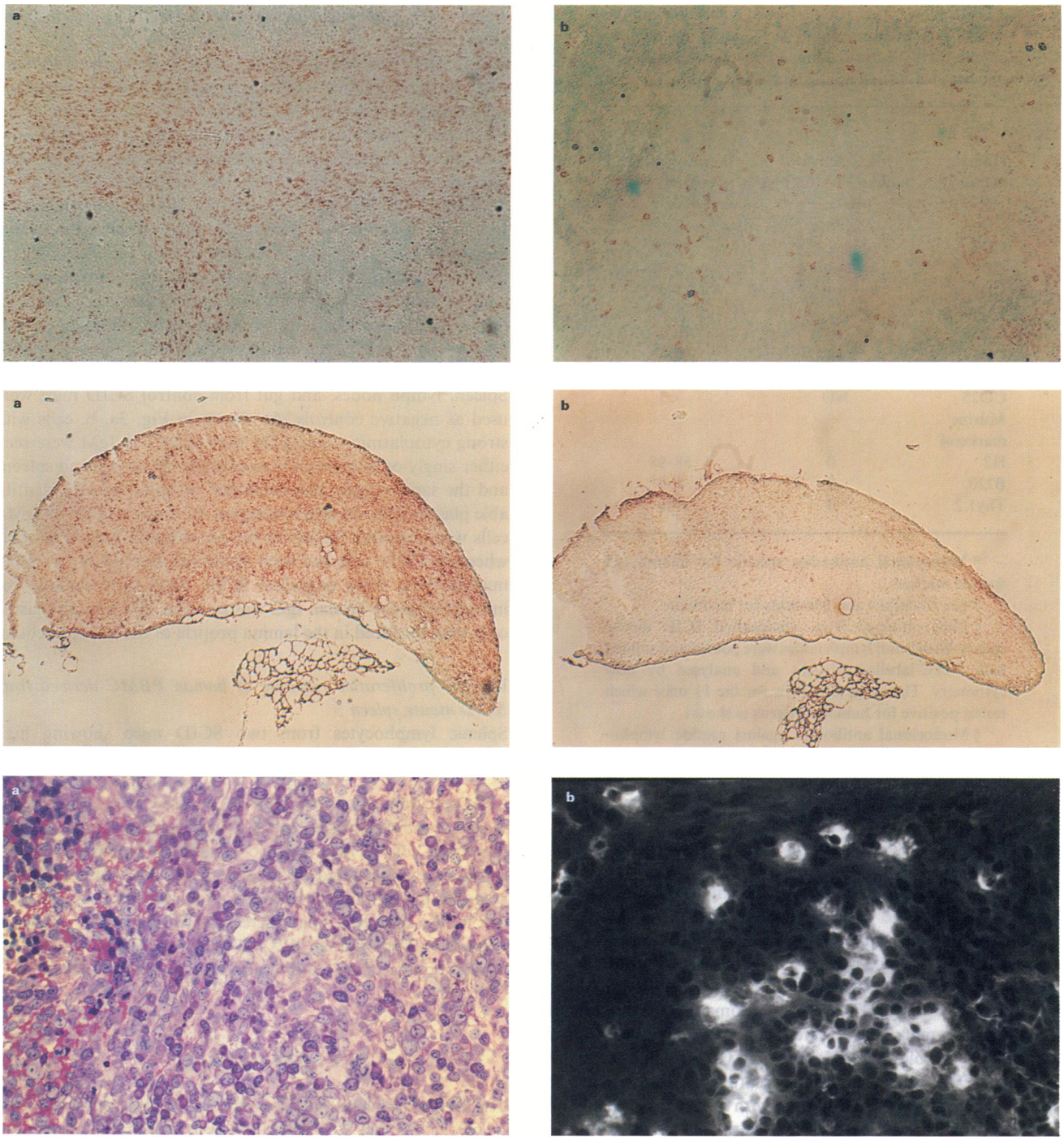

Fig. 1. Demonstration of human T cells in xenografted SCID mouse spleen. The SCID mice were killed 4 weeks after i.p. injection of 50×10^6 human peripheral blood mononuclear cells (PBMC) preactivated *in vitro* in a 4-day mixed lymphocyte culture (MLC). The tissues were fixed and stained with biotinylated CD2 MoAb and B220 MoAb. The MoAbs were detected by a streptavidin-peroxidase reaction (Zymed Histostain DS). (a) SCID mouse spleen stained with CD2 shows perivascular areas of positive cells ($\times 40$). (b) SCID mouse spleen (same organ as (a)) stained with B220 MoAb ($\times 100$).

Fig. 2. Demonstration of human T cells in xenografted SCID mouse lymph node. Frozen sections of lymph node tissue were obtained from a SCID mouse 4 weeks after injection of 50×10^6 human peripheral blood mononuclear cells (PBMC) preactivated *in vitro* in a 4-day mixed lymphocyte culture (MLC). The tissues were stained with biotinylated CD2 and B220, followed by streptavidin-peroxidase, as in Fig. 1. (a) SCID mouse cervical lymph node stained for human T cells with CD2 shows many scattered positive cells without obvious histological organization ($\times 40$). (b) The same lymph node stained with B220 MoAb shows few positive cells ($\times 40$).

Fig. 3. Demonstration of human plasma cells in xenografted SCID mouse lymph node; frozen sections and paraffin-embedded section of lymph node from a SCID mouse 4 weeks after i.p. injection of 50×10^6 human peripheral blood mononuclear cells (PBMC) that had been preactivated in an *in vitro* 4-day mixed lymphocyte culture (MLC). (a) Paraffin section of SCID mouse cervical lymph node stained with haematoxylin and eosin. Plasma cells are abundant and characterized by eccentric nuclei and a dark cytoplasm ($\times 100$). (b) Frozen section of the same lymph node stained for human intracytoplasmic IgG using FITC-conjugated anti-IgG (heavy chain) ($\times 100$).

lymphoid tissues (Table 2), and murine immunoglobulin levels in the serum did not increase.

It has been suggested that murine NK cells could play a role in human cell engraftment [16]. However, variability in NK cell functions are unlikely to be the sole explanation for differences in engraftment. We found little variation in NK cell activity between individual SCID mice (data not shown). Also, recent data show that the abrogation of murine NK cells in xenografted SCID mice enhances murine T and B cell lymphopoiesis but has little effect on the engraftment of human lymphocytes [19]. Engraftment of human thymic stroma in SCID mice was improved by NK cell depletion, but the thymic stroma was colonized with murine cells, not by human T cells [19].

Our findings of human immunoglobulins in the serum of most xenotransplanted animals is consistent with previous reports [5,7,15]. However, very few or no $CD19^+$ lymphocytes were detected in the animals at 3–4 weeks after xenotransplantation. This suggests that in our system, the $CD19^+$ cells either differentiate into plasma cells or did not survive long-term in SCID lymphoid tissues.

Several studies have shown Epstein–Barr virus (EBV)-lymphoproliferative disease in SCID mice xenografted with cells from EBV^+ donors [5,20,21]. Mosier *et al.* found increasing numbers of B cells and EBV^+ lymphoproliferation in 63% of SCID mice xenografted with $10–90 \times 10^6$ PBMC from EBV^+ donors [5]. Even though the donors in our study were EBV^+, B cell tumours did not arise, suggesting that the *in vitro* preactivation of the PBMC may have prevented their development. The findings of engrafted macrophages in our study are similar to the data of Mosier *et al.* but different from Tary-Lehman & Saxon who found no evidence of engraftment of human monocytes [15].

Consistent with previous studies, most of the human cells recovered from the SCID mice at 3–4 weeks were T cells [7,15]. The human T cells were localized to murine spleen, lymph nodes, and gut mucosa. No definitive homing pattern of human T cells to the SCID lymph nodes or gut mucosa was seen. In the murine spleens, human T cells had a perivascular distribution. These studies do not adequately address the question of the homing capability of the surviving human lymphocytes. However, they suggest that important differences exist between murine and human homing signals.

Relatively large numbers of human T cells were recovered from the spleens of some animals. Functional testing showed no response to tetanus toxoid, candida antigen, or streptococcal antigen. Very low reactivity was detected against the original MLC stimulators. These results are similar to those of Tary-Lehman & Saxon [15] who found no T cell functional activity immediately after removal from the animals. This lack of functional activity could be due to either the selective survival of T cells which do not recognize the above antigens or the induction of unresponsiveness of human T cells by the murine microenvironment. Continuous antigenic stimulus might be necessary for maintaining human T cell function. Our recent studies suggest that human $\gamma\delta$ T cells can proliferate in SCID mice in response to repeated specific antigenic stimulation. Experiments to define the precise requirements for this persistent functional activity of human T cells in SCID mice are in progress.

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